ONLINE METHODS

Subjects. A full description of study cohorts is in the Supplementary Note and Supplementary Tables 1 and 2.

Covariate Data. Age-of-onset was available from some cohorts [Alzheimer' Disease Center (ADC), Translational Genomics Research Institute series 2 (TGEN2), National Institute on Aging Late-onset AD (NIA-LOAD), Multi-Institutional Research on Alzheimer's Genetic Epidemiology (MIRAGE), Adult Changes in Thought (ACT), Multi-Site Collaborative Study for Genotype-Phenotype Associations in Alzheimer's Disease (GenADA), University of Pittsburgh (UP), and the Rush University Religious Orders Study/Memory and Aging Project (ROS/MAP)], while for others, only age at ascertainment [Washington University (WU), ADNI], age at diagnosis [Mayo clinic (MAYO)], or a combination of both age at ascertainment and age at death was available [a subset of autopsy-confirmed samples in the University of Miami/Vanderbilt University/Mt. Sinai School of Medicine (UM/VU/MSSM) cohort]. For subjects with autopsy-confirmed diagnosis and no clinical diagnosis, the age at diagnosis was equated to the age at death. For all studies, the age used for CNEs was the age of last exam or age at death. Case and CNE subjects with age at symptom onset or age at death less than 60 were excluded from analysis. We restricted our association analyses to Caucasians of European ancestry because there were insufficient subjects from non-Caucasian groups to obtain meaningful results.

Genotyping, data cleaning, and imputation. Genotypes were from either Illumina or Affymetrix high-density SNP microarrays (Supplementary Table 3). Genotype data were cleaned by applying minimum call rates (95% and 98%) and minimum minor allele frequencies (0.02 and 0.01) for cohorts genotyped on Affymetrix and Illumina chips, respectively. SNPs not in Hardy-Weinberg equilibrium ($P < 10^{-6}$) were excluded. Subjects where the gender was mis-specified were identified by analysis of X-chromosome SNPs using PLINK[25]. For cohorts genotyped on multiple chips (MIRAGE, UM/UV/MSSM), genotype and sample quality thresholds were applied within subsets of individuals genotyped on each chip. For all other cohorts, quality thresholds were applied per cohort. Relationships among individuals in the family-based cohorts (MIRAGE, NIALOAD) were confirmed by pair-wise genome-wide estimates of proportion identity-by-descent (IBD) using PREST software[26]. Any discrepancies identified were reviewed in light of available clinical and pedigree data to determine the most likely relationship consistent with a proportion of IBD, and any remaining scenarios were excluded from analysis. Latent relatedness in the case-control cohorts were identified by proportion IBD using PLINK software[25,27]. Both of each pair of identical samples by IBD ($\hat{\pi} > 0.99$) were dropped, while one subject was selected from each related pair ($0.4 \geq \hat{\pi} > 0.90$), prioritizing non-missing case/control status and then higher call rate in selection. Duplicate enrollments among studies (Supplementary Table 4) were identified using proportion of IBD in a genotyped dataset including all cohorts, where pairs with $\hat{\pi} > 0.95$ were considered duplicate

enrollments. Duplicates with discordant case/control status by study were dropped from both studies, while those with concordant status were included in only one cohort, selected according to a pre-determined priority list of cohorts, which considered genotype data, phenotype data, and the type of cohort. Genome-wide imputation was performed per cohort using MaCH software[28] with HapMap phase 2 (release 22) CEPH Utah pedigree (CEU) reference haplotypes and genotype data passing quality control as inference. Imputation quality was determined as R^2 and only SNPs imputed with $R^2 \geq 0.50$ were included in analysis.

***APOE* genotyping.** *APOE* genotypes were determined for the ADC, ACT, NIA-LOAD, UM/VU/MSSM, MAYO, and GenADA cohorts using SNPs rs7412 and rs429358, for the MIRAGE cohort using the Roche Diagnostics LightCycler® 480 instrument (Roche Diagnostics, Mannheim, Germany)[29] LightMix® Kit ApoE C112R R158 (TIB MOLBIOL), for TGEN2, ADNI, UP and WU cohorts by pyrosequencing[30], or restriction fragment length polymorphism analysis[31 32] and for ROSMAP by high-throughput sequencing of codons 112 and 158 in *APOE* by Agencourt Bioscience Corporation (Beverly, MA).

Meta-analysis. Presence of intra-study population substructure was evaluated separately by cohort in a two-step process that first removes outliers before estimating population substructure within the remaining population. For the first step, either the STRUCTURE software package[33-34] (UM/VU/MSSM, MIRAGE) or the *smartpca* script in EIGENSTRAT[35] (remaining cohorts) was used to remove outliers and/or confirm self-reported ethnicity after filtering to remove SNPs

in pair-wise linkage disequilibrium (LD). In the second step, we used EIGENSTRAT[35], often a second time, to estimate principal component loadings (PCs) for inclusion in association analysis. For each study, the first two, three or four estimated PCs were identified for inclusion as covariates in association analysis (Supplementary Table 3). Outlier detection for the ADC, TGEN2, GenADA, ACT, ADNI, ROS/MAP, OHSU, UP, WU and MAYO cohorts was evaluated by comparison to the HapMap 3 CePH (CEU) population. EIGENSTRAT analyses of family cohort data (NIA-LOAD and MIRAGE) used a sample of unrelated individuals to fit principal components after outliers with respect to European-American ancestry were removed.

Genotyped and imputed SNP data passing quality control were tested for association with AD in each dataset using logistic generalized linear model (GLM) for case-control analysis, and logistic generalized estimating equations (GEE) for family-based cohorts in R[36-38]. All analyses assumed an additive genetic model, coding genotyped SNPs by the number of minor alleles (0, 1, or 2) and imputed SNPs by the posterior probability of the minor allele (range 0 to 2). Primary association analyses were adjusted for population substructure (baseline model).

SNP association results for each dataset were meta-analyzed using the inverse variance method implemented in the software package METAL[39]. The meta-analysis *P*-value was estimated by the summarized test statistic, after applying a genomic control within each

individual study. Heterogeneity among odds ratios in meta-analysis was assessed using Cochran's Q and I^2 statistic [40-41].

Regional association plots were prepared for the most strongly associated SNPs in the *CR1*, *BIN1*, *CD2AP*, *EPHA1*, *CLU, MS4A4A/6A*, *PICALM*, *ABCA7*, and *CD33* genes using the gene locations from UCSC Genome browser (hg19, GRCh37, Feb 2009 release) and SNP locations from corresponding dbSNP build 131. Estimates of linkage-disequilibrium were calculated with the FUGUE software[42] using HapMap phase 2 (release 24, CEU) genotype data and build 131 SNP positions. Forest plots of study-specific effects and analysis results are presented for the same set of SNPs using *rmeta* package in R.

Joint analysis. Population substructure across studies was performed in a combined dataset using the set of SNPs genotyped in all study cohorts. After filtering SNPs with pairwise LD (r^2) < 0.20, 31,310 SNPs were evaluated using EIGENSTRAT. The top three principal components from EIGENSTRAT were used as covariates in the joint analysis for association, in addition to an adjustment for site-specific effects using dummy variables for each cohort. SNP associations with AD affection status were examined in a pooled analysis of subjects from all cohorts, excluding SNPs missing from one or more individual dataset or with genotypes available on fewer than 98% of individuals overall. In total, 2,312,972 directly genotyped or imputed SNPs common to all datasets were tested for association in 8,309 cases and 7,366 CNEs, including 3,489 individuals in family datasets using GEE analyses in R. Joint analyses of the baseline

model, full model and models evaluating robustness to APOE include as covariates the PCs from inter-study and intra-study population substructure and a dummy covariate for cohort-specific effects. Genomic inflation factors for the discovery joint analysis in the basic and extended models of covariate adjustment were 1.05 and 1.04, respectively (Supplementary Table 3), similar to those from meta-analysis.

Secondary analysis. Association results in regions yielding at least one SNP with $P < 10^{-6}$ (follow-up SNPs) were further evaluated for robustness to APOE ε4 carrier status in analyses stratified according to presence or absence of *APOE* ε4 and an interaction analysis including effects for SNP, *APOE* ε4, and their interaction. In addition, we examined the *EXOC3L2* region in chromosome 19 previously reported as independent of *APOE* genotype[2] in a full model including covariates for age at onset/exam, gender and the dosage of *APOE* ε4 alleles.

Internal and external replication analyses. SNPs attaining a $P \leq 1 \times 10^{-6}$ for association with LOAD in the Discovery cohort were evaluated in five independent datasets (ADC3, OHSU, MAYO, ROS/MAP, UP) consisting of 3,531 cases and 3,565 CNEs using the same analytical approaches as described above. Replication was performed using both *meta*- and joint analysis. The datasets included in discovery and replication analyses are summarized in Supplementary Tables 1 and 2. Following internal replication, an external replication cohort was sought to evaluate the most strongly associated SNP in each of four novel genes (*CD2AP*/rs9349407,

EPHA1/rs11767557, *ARID5B*/rs2588969, and *CD33*/rs3865444) for which results did not met genome-wide significance ($P_M > 5 \times 10^{-8}$ and $P_J > 5 \times 10^{-8}$) in the combined discovery and replication datasets (Stages 1 + 2) was sought by meta-analysis of summarized results from five independent external datasets generously provided by the GERAD Consortium[1], the EADI Consortium[3], and the CHARGE Consortium[2]. After removing subjects recognized as part of the ADGC cohorts (Hollingsworth *et al.*[12]), the sample included 7,650 AD cases and 25,839 normals. These datasets were analyzed using meta-analysis as described above for the Stage 1 and 2 datasets. Results from Stages 1, 2, and 3 were likewise by meta-analysis as described above.